

MCS₁

	Acc651/Kpn1			
	Smal	/XmaI AgeI		
	BamHI		Start of mOrange2	
261	AGAGGATCCC	CGGGTACCGG	TATGGTGAGC	
	TCTCCTAGGG	GCCCATGGCC	ATACCACTCG	

MCS₂

		<u> </u>	
	NotI	EcoRI	
991	GAGCGGCCGC	GACTCTAGAA	TTCCAACTGA
	CTCGCCGGCG	CTGAGATCTT	AAGGTTGACT

pmOrange2 Restriction Map and Multiple Cloning Sites (MCS).



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Description

pmOrange2 is a prokaryotic expression vector that encodes mOrange2, a mutant fluorescent protein derived from mOrange (1) that has been optimized for photostability. The excitation and emission maxima of mOrange2 are 549 nm and 565 nm, respectively.

The vector's mOrange2 coding sequence is flanked by separate and distinct multiple cloning sites (MCS1 and MCS2) that make it easy to excise the gene for use in other cloning applications. Alternatively, the mOrange2 coding sequence can be amplified by PCR. In *E. coli*, mOrange2 is expressed from the *lac* promoter as a fusion with several amino acids, including the first five amino acids of the LacZ protein. Note, however, that if the mOrange2 coding sequence is excised using a restriction site in the 5' MCS, the resulting DNA fragment will encode only the mOrange2 protein (without the additional amino acids that are expressed using the *lac* promoter). In pmOrange2, the entire mOrange2 expression cassette is supported by a pUC19 backbone, which contains a high copy-number origin of replication and an ampicillin resistance gene for propagation and selection in *E. coli*.

(PR963265; published 6 July 2009)

pmOrange2 **Vector Information**

Use

pmOrange2 is primarily intended to serve as a source of mOrange2 cDNA. The flanking MCS regions make it possible to excise the mOrange2 coding sequence and insert it into other vector systems. The vector can also be used to express the mOrange2 protein in bacteria.

For Western analysis, either the Living Colors® DsRed Polyclonal Antibody (Cat. No. 632496) or the DsRed Monoclonal Antibody (Cat. Nos. 632392 and 632393) can be used to detect the mOrange2 protein.

Location of features

- *P_{lac}* (*lac* Promoter): 95–178
- 5' MCS (5' multiple cloning site): 264–281
- mOrange2: 282–989
- 3' MCS (3' multiple cloning site): 993-1013
- Amp^r (ampicillin resistance gene: β-lactamase): 1538–2398
- pUC origin of replication: 2546–3188

Propagation in *E. Coli*

- Recommended host strain: DH5α[™]
- Selectable marker: plasmid confers resistance to ampicillin (50 µg/ml) in E. coli hosts.
- E. coli replication origin: pUC
- Copy number: high
- Plasmid incompatibility group: pMB1/ColE1

Excitation and emission maxima of mOrange2

- Excitation maximum = 549 nm
- Emission maximum = 565 nm

References

1. Shaner, N. C., et al. (2008) Nature Methods. 5(6):545-551.

Note: The vector sequence was compiled from information in the sequence databases, published literature, and other sources, together with partial sequences obtained by Clontech. This vector has not been completely sequenced.

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